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SHORT COMMUNICATION

Bituminaria plumosa (Fabaceae), a critical species of the Croatian flora

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Background – *Bituminaria* is a small genus of the family Fabaceae that belongs to the tribe Psoraleeae distributed both in the Mediterranean and Macaronesian region. In the framework of taxonomic research on this genus, an unresolved species described from Croatia, and previously attributed to *Psoralea*, is here restored and properly transferred to *Bituminaria*, thus proposing a new combination.
Methods – Field investigations, herbarium specimens, morphological analyses and literature were used for a correct identification of this species and its comparison with the other known taxa of the genus *Bituminaria*.
Results – *Bituminaria plumosa* (Rchb.) Bogdanović, C.Brullo, Brullo, Giusso & Ljubičić (= *Psoralea plumosa* Rchb.), originally described from Istria (North Croatia), is recognized as a distinct species of this peculiar genus. Considerations on its morphological features, seed testa micro-sculptures, ecology, chorology, taxonomical relationships, as well as a new and detailed iconography are provided. This species is morphologically well distinct from the other known species of the genus in having a densely villous indumentum, with very long hairs, leaf petiole very short, leaf blades ovate to ovate-lanceolate, long aristate, corolla purplish-pink, standard elliptical, long pistil, and large seeds.

Key words – Analytic key, *Bituminaria*, *Bituminaria plumosa*, Croatia, Mediterranean, new combination, *Psoralea*, taxonomy.

INTRODUCTION

Bituminaria Heist. ex Fabr., especially for its inflorescence and pod, is a peculiar genus of the family Fabaceae occurring in the Mediterranean, Black Sea area, and Canary Islands. This genus was included by Rydberg (1919, 1928) in the tribe Psoraleeae, which currently groups nine natural genera widely spread in the Old and New World (Grimes 1990, Kirkbride et al. 2003, Lewis et al. 2005, Egan & Crandall 2008a). According to recent phylogenetic studies (Egan & Crandall 2008a, 2008b, Dludlu 2010, Dludlu et al. 2013, Toksoy et al. 2015), the genus *Bituminaria* has to be considered a distinct monophyletic genus closely related to *Cullen* Medik. and *Otholobium* C.H.Stirt. Currently, only few species are recognized within *Bituminaria* (Stirton 1981a, 1981b, Greuter 1986, Greuter et al. 1989, Feinbrun-Dothan & Danin 1998, Boulos 1999, Minissale et al. 2013, Bacchetta et al. 2014, Giusso del Galdo et al. 2015). The species nowadays included in *Bituminaria* were formerly ascribed to the genus *Psoralea* L., to which a wider circumscription was given. Those currently recognized within *Bituminaria* are: *B. bituminosa* (L.) C.H.Stirt. (type species of the genus), *B. morisiana* (Pignatti & Metlesics) Greuter, *B. flaccida* (Náb.) Greuter, *B. basaltica* Miniss., C.Brullo, Brullo, Giusso & Sciandr. and *B. kyreniae* Giusso, C.Brullo, Brullo, Cambria & Miniss., all belonging to the subgen. *Bituminaria*, and lastly *B. acaulis* (Steven ex M.Bieb.) C.H.Stirton, included in the subgenus *Christevenia* Barneby ex C.H.Stirton by Stirton (1981a). Previously, several taxa at specific or infraspecific level have been described by several authors (Goüan 1773, Reichenbach 1832, Zohary 1972, Méndez et al. 1990, Khokhrjakov 1997) and ascribed to *Psoralea* or *Bituminaria* (cf. Minissale et al. 2013). Therefore, basing both on literature and our studies, *B. bituminosa* must be treated as a species complex, which deserve further in-depth taxonomic and phylogenetic studies, as already suggested by Grimes (1990).

In the framework of a research aiming at improving the knowledge on this poorly investigated genus of the Mediterranean flora, we studied a critical species, described by Reichenbach (1832) from Dalmatia (Croatia) and named *Psoralea plumosa*. Later, Reichenbach & Beck de Manna-

getta (1903: Tav. 140 I) published a fine and colored illustration of this plant, but it was considered to represent a variety of *Psoralea bituminosa*, highlighting some of its most relevant diacritical features. This taxon was quoted by several authors (Grenier & Godron 1848, Nyman 1854–1855, 1878, Willkomm & Lange 1880, Cesati et al. 1884, Rouy 1899, Fiori & Paoletti 1900, Hayek 1927) who treated it at specific or infraspecific level, recording this legume from many other Mediterranean countries. Based on our study of herbarium specimens and field investigations, it is clear that the aforesaid authors used (as it is quite evident from the diagnostic descriptions reported in their floras) inappropriate morphological characters for the distinction of the taxon, thus leading to erroneous identifications.

In order to clarify the taxonomic, nomenclatural and phytogeographical aspects of this peculiar and still poorly known legume of the Mediterranean flora, in-depth investigations were carried out mainly in the area from which this plant was originally collected, as well as in the surrounding territories. Furthermore, such surveys allowed to shed a light on the ecology, distribution range, macro- and micro-morphology of this critical legume. This allowed to establish that *P. plumosa* is a taxon to be treated at specific level, distributed all over the Adriatic side of Croatia, and that it should be moved to the genus *Bituminaria.*

MATERIAL AND METHODS

The morphological analyses on *Bituminaria plumosa* were carried out on herbarium specimens (c. 30) and several living plants coming from Istria and other localities of Croatia, while for the other species of *Bituminaria* the herbarium material recorded by Minissale et al. (2013) and Giusso del Galdo et al. (2015) was used. In addition, living plants included in this study are cultivated in the Botanical Garden of Catania, and are originated from seeds collected all over the Mediterranean (e.g. Spain, Baleares, Corsica, Sardinia, Sicily, Italy, Croatia, Greece, Cyprus, Turkey, Israel, Jordan, Tunisia, Morocco, Canary Islands, etc.). Finally, herbarium material preserved in the following herbaria was also studied: BEOU, BP, BR, CAT, CNHM, HEID, LJM, TUB, ZA, ZAGR, ZAHO, ZT, W and WU (abbreviations are according to Thiers 2015). Seed testa micro-morphology was studied on ten mature and dried seeds using a scanning electron microscope (SEM) Zeiss EVO LS10, according to the protocol reported by Stork et al. (1980), while terminology of the seed coat sculpturing follows Barthlott (1981) and Gontcharova et al. (2009).

TAXONOMIC TREATMENT

Bituminaria plumosa (Rchb.) Bogdanović, C.Brullo, Brullo, Ljubičić & Giusso, **comb. nov.** – *Psoralea plumosa* Rchb., Flora Germanica Excursoria: 869. 1832 (Reichenbach 1832). – *Psoralea bituminosa* L. var. *plumosa* (Rchb.) Rchb. fil. (Reichenbach in Reichenbach & Beck de Mannagetta 1903: 91). – *Psoralea bituminosa* L. subsp. *plumosa* (Rchb.) Nyman (Nyman 1878: 188). – Type: In Dalmatien an steinigen Orten am Meere, *Welden* (not found, likely destroyed or missing). Neotype (Icon.), **designated here**: Tab. 140 I, MMCXCI in Reichenbach & Beck de Mannagetta (1903). Epitype, **designated here**: Hrvatska, Istra, Kavran, rub šume, uz cestu, 13 Jun. 2014, *Bogdanović & Ljubičić* s.n. (epi-: ZAGR, barcode ZAGR-37771, isoepi-: CAT, ZAGR, barcode ZAGR-37773).

Perennial herb, 60–90(–150) cm tall, woody and branched at the base, green, densely villous, with hairs subpatent, 0.5–1.5 mm long, slightly smelling of pitch. Stems numerous, erect-ascending, rigid, striate. Stipules lanceolate-ensiform, 7–15 mm long, adnate to the petiole. Leaves petiolate, digitately 3-foliate, leaflets densely villous on both surfaces and densely ciliate at the margin, the central one larger and long petiolate, the lateral ones shorter and subsessile or shortly petiolate; basal leaves smaller, with petiole 1.5–3(–4) cm long and leaflets ovate, 12–20 × 6–12 mm, acute, rounded and apiculate at the apex, with mucro long 1–1.5 mm, 3–5-nerved; the cauline ones much larger, reducing progressively upwards, with petiole 1.5–6 (–8) cm long and leaflets ovate to ovate-lanceolate, 18–65 × 8–28 mm, 5–8-nerved. Raceme capitate, 1.8–3 cm long, 15–25(–30)-flowered; bracts, calyces and upper part of peduncle covered by white and black hairs; peduncles (6–)8–21 cm long, much longer than the leaf. Bracts 5–15 mm long, 1–3-toothed. Calyx 15–16 mm long, 10-nerved with unequal triangular-subulate teeth; tube 6–7 mm long; lower tooth 8–9 mm long; lateral teeth 6–7 mm long. Corolla longer than calyx; standard purplish-pink upwards, whitish-pink below, elliptical, emarginate, 19–20 × 7–7.5 mm; wings pale purplish-pink, 18–18.5 mm long, with limb 4–4.2 mm wide; keel pink, 12–13 mm long, with limb 2–2.2 mm wide, with macula dark purple at the top. Staminal tube 11–12 mm long; anthers 0.4–0.5 mm long, apiculate. Pistil 13–14 mm long, hairy in the ovary; stigma smooth, ciliate at the base. Pod indehiscent, included in the calyx, 16–18 mm long (beak included), densely covered in the corpus by setaceous hairs, 1–3 mm long, mixed to some rigid black prickles; beak flat, slightly falciform, 10–12 mm long, sparsely hairy and ciliate at the margin. Seed adherent to pericarp, laterally compressed, subreniform, 5.5–6 × 4.5–4.8 mm, blackish-brown. Figs 1, 2 & 3.

Seed and pod micro-morphology – Several authors previously carried out research on the seed testa ornamentation of Fabaceae (see Murthy & Sanjappa 2002, Kirkbride et al. 2003, Salimpour et al. 2007, Bacchetta & Brullo 2010, Fawzel 2011, Gandhi et al. 2011, Brullo et al. 2011, 2013), thus emphasizing its systematic and phylogenetic importance in genera or critical groups. In particular, these micro-sculptures may add meaningful information chiefly useful for the identification of a given species. As concerns *Bituminaria*, SEM investigations of the seed testa of *B. bituminosa*, *B. basaltica* and *B. kyreniae* were performed by Minissale et al. (2013) and Giusso del Galdo et al. (2015). Our examination of the seed coat of *B. plumosa* (fig. 4) reveals that the micro-sculptures occurring on the testa are very different from those of the aforesaid species. In particular, the seed coat of *B. plumosa* is characterized by a fine and often lacerate reticulum bounding the single cells, which are irregularly polygonal and 11–14 μm wide. The anticlinal walls are slightly grooved, rugose and sometimes slightly lacerate, while the periclinal ones are slightly depressed at the centre with epi-

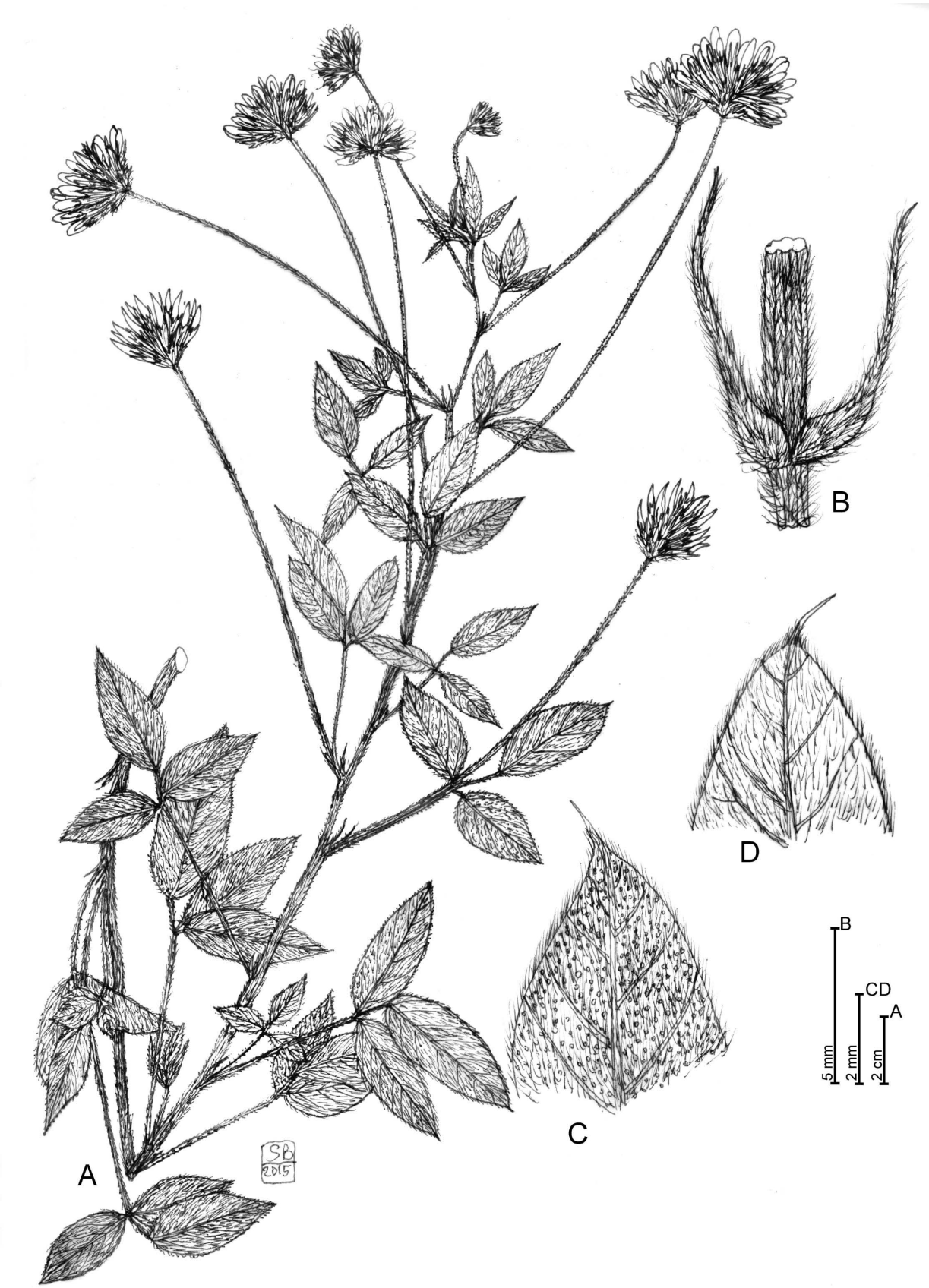

Figure 1 – *Bituminaria plumosa*: A, habit; B, stipule; C, leaflet apex (abaxial side); D, leaflet apex (adaxial side). Illustration by S. Brullo based on material coming from Kavran, *Bogdanović & Ljubičić* s.n. (CAT, ZAGR).

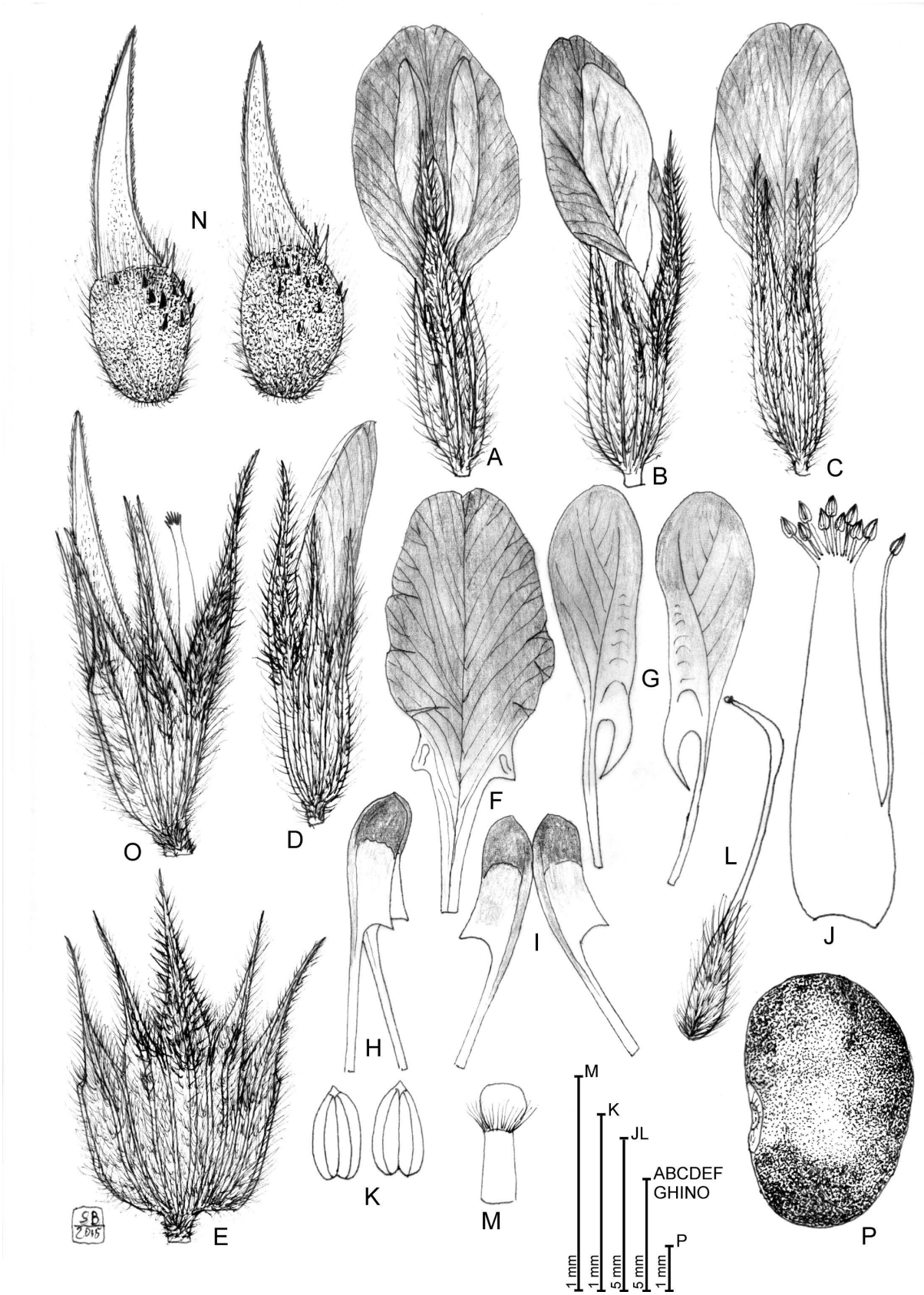

Figure 2 – Diagnostic features of *Bituminaria plumosa*: A, flower (ventral view); B, flower (lateral view); C, flower (dorsal view); D, bud; E, open calyx; F, standard; G, wings; H, keel (lateral view); I, open keel; J, staminal tube; K, anthers; L, pistil; M, stigma; N, pods; O, pod with calyx; P, seed. Illustration by S. Brullo material coming from Kavran, *Bogdanović & Ljubičić* s.n. (CAT, ZAGR).

Key to known species of *Bituminaria* subgen. *Bituminaria*

1. Cauline leaflets linear; corolla pure white, 11–13 mm long, subequalling the calyx; staminal tube 7–8 mm long.....*B. basaltica*
1'. Cauline leaflets not linear, corolla whitish-pink to blue-violet 15-24 mm long, longer than calyx; staminal tube 9–15 mm long.....2
2. Raceme 5–8-flowered.....3
2'. Raceme 10–30-flowered.....4
3. Calyx 9–11 mm long; corolla whitish-pink; pod (included beak) maximum 10 mm long.....*B. flaccida*
3'. Calyx 12–16 mm long; corolla blue-violet to violet; pod (included beak) 16–22 mm long...*B. kyreniae*
4. Stems and leaves densely villous; corolla purplish-pink, with standard elliptical; pod max 18 mm long (including beak).....*B. plumosa*
4'. Stems and leaves hirsute to subglabrous; corolla white-violet to blue-violet, with standard ovate-elliptical, ovate-lanceolate or oblanceolate-spathulate; pod up to 26 mm long (including beak).....5
5. Leaflets sparsely hairy to glabrous, max 42 mm long; raceme 3–4.5 cm long; corolla white-violet, with standard ovate lanceolate, obtuse.....*B. morisiana*
5'. Leaflets hirsute, up to 90 mm long; raceme 2–2.8 cm long; corolla blue-violet, with standard ovate-elliptical, emarginate.....*B. bituminosa*

dermis slightly striate-rugose (fig. 4B). Some differences occur also in the pod indumentum, since the surface of the hair covering the corpus of *B. plumosa* is finely rugose and the hairs have a longitudinal furrow widened at the foot with a basal diameter of c. 27 µm (fig. 4D).

Phenology – Flowering from April to early June, fruiting from June to August.

Distribution – In the protologue, Reichenbach (1832) provided just a vague indication as concerns the location of this species, as follows: "*In Dalmatia on stony places near the sea*". Afterwards, Reichenbach & Beck de Mannagetta (1903) recorded it from Istria in two precise locations, namely Cavrano (nowadays Kavran) and between Altura (nowadays Valtura) and Carnizze (nowadays Krnica). Actually, both stands are very close and situated in the eastern side of the southern point of the Istrian Peninsula, where it still exists as recently verified by us. Based on herbarium surveys, as well as on field investigations carried out in the several Mediterranean countries, this species occurs, apart from SE

Figure 3 – Habit and inflorescence detail of *Bituminaria plumosa* from Kavran (Photographs: S. Bogdanović).

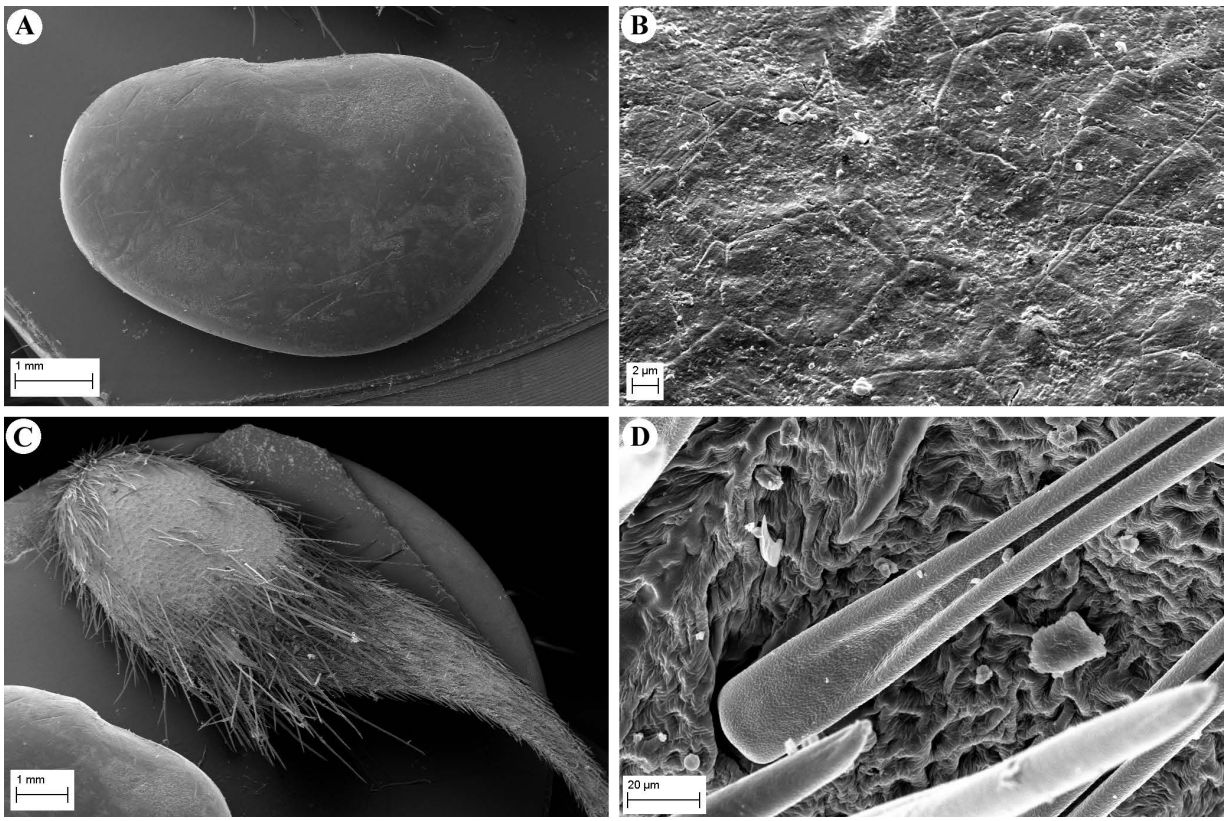

Figure 4 – Scanning Electron Micrographs (SEM) of *Bituminaria plumosa*: A, seed (x15); B, seed coat (x2500); C, pod (x10); D, detail of pod hairs (x700). Based on material coming from Kavran, *Bogdanović & Ljubičić* s.n. (CAT).

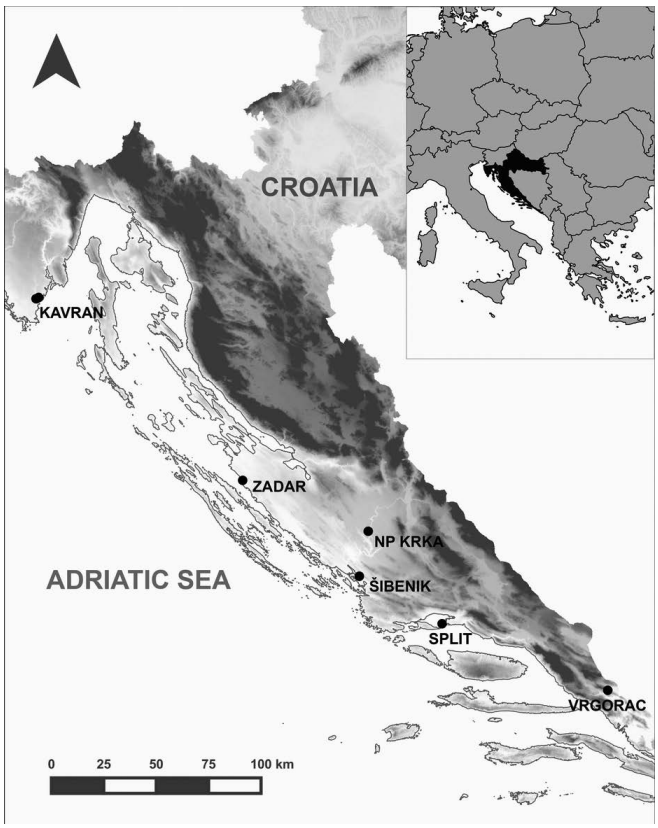

Figure 5 – Distribution map of *Bituminaria plumosa* in Croatia.

Istria, also in many other localities along the Adriatic coast of Dalmatia (fig. 5).

Habitat and ecology – Based on field observations, *B. plumosa* grows at the margin of *Quercus ilex* woodlands (in Istria) and /or paths and roads within semi-natural phytocoenoses.

Taxonomic remarks – According to the literature and herbarium investigations, as well as morphological observations carried out on several living plants collected in many Mediterranean localities, *B. plumosa* is well differentiated from the other known species of this genus (see table 1). In particular, the main diagnostic features are represented by a dense villous indumentum covering the whole plant, leaflets ovate to ovate-lanceolate, provided with a long mucro, corolla purplish-pink with elliptical standard and longer pistil (fig. 3). Besides, there are relevant differences also in the seed coat sculptures, especially if compared with those of *B. bituminosa*, *B. basaltica* and *B. kyreniae*, already surveyed by Minissale et al. (2013) and Giusso del Galdo et al. (2015). Table 1 provides an overview of these diagnostic features and their comparison with the other taxa belonging to *Bituminaria* subg. *Bituminaria.*

In order to verify the existence of exsiccata to be used for the typification of *Psoralea plumosa* Rchb., we carried out an in-depth research in the herbaria of several Botanical Museums (BEOU, BP, BR CNHM, HEID, LJM, TUB, ZA, ZAGR, ZAHO, ZT, W and WU), where its presence would be expected, but we were fully unsuccessful. Therefore, it

Table 1 – Main diagnostic characters of the species belonging to *Bituminaria* subgen. *Bituminaria*.

	B. plumosa	*B. bituminosa*	*B. basaltica*	*B. morisiana*	*B. flaccida*	*B. kyreniae*
Stem habit	erect-ascending	erect (rar. prostrate)	erect to ascending-erect	erect-ascending	ascending to procumbent	erect-ascending
Plant height (cm)	up to 150	up to 150	up to 60	up to 60	up to 40	up to 50
Stipule length (mm)	7–15	4–15	3–6	8–11	2–7	4–10
Leaf indumentum (abaxial side)	densely villous	hirsute	hirsute	sparsely hairy	hirsute	sparsely hairy
Leaf indumentum (adaxial side)	densely villous	hirsute	glabrous to subglabrous	glabrous to subglabrous	hirsute	glabrous to subglabrous
Leaf petiole length (cm)	1.5–6(–8)	1.5–15	4–10	1.5–20	1–6.5	3–12
Basal leaflet shape	ovate	rounded-elliptical to lanceolate	rounded-elliptical to linear-lanceolate	ovate-lanceolate to elliptical	suborbicular to obovate	ovate to lanceolate
Cauline leaflet shape	ovate-lanceolate	elliptical to lanceolate	linear	ovate-lanceolate to lanceolate	obovate to linear-lanceolate	ovate to lanceolate
Cauline leaflet mucro (mm)	1–1.5	0.3–0.5	0.5–0.8	0.4–0.5	0.4–0.8	0.3–0.5
Leaflet length (mm)	12–65	3–90	8–55	27–42	4–20	12–60
Leaflet width (mm)	6–28	6–30	2–15	6–20	3–12	4–20
Peduncle raceme length (cm)	(6)8–21	8–22	10–16	4–12	2–9	5–20
Raceme shape	capitate	capitate	capitate	capitate to ovoid	capitate	sub-capitate
Raceme length (cm)	1.8–3	2–2.8	1–1.6	2.5–4.5	1.7–2	2–2.8
Raceme (number of flowers)	(10–)15–25(–30)	15–30	6–12(–16)	10–25	5–8	5–10
Bract length (mm)	5–15	6–15	6–8	6–9	3–5	5–12
Calyx length (mm)	15–16	14–18	10–13	15–18	9–11	12–16
Calyx tube length (mm)	6–7	6–7	4–5	5–7	5–6	5–8
Calyx lower tooth length (mm)	8–9	7–12	6–9	7–10	5–6	7–11
Calyx lateral teeth length (mm)	6–7	7–9	4–6	6–8	4–5	5.5–9
Corolla (colour)	purplish-pink	blue-violet	white	white-violet	whitish-pink	blue-violet to violet
Standard shape	elliptical	ovate-elliptical	spathulate	ovate-lanceolate	ovate-elliptical	oblanceolate-spathulate
Standard apex	emarginate	emarginate	rounded to obtuse	obtuse	obtuse	usually rounded
Standard length (mm)	19–20	15–20	11–13	18–23	16–18	16–24
Standard width (mm)	7–7.5	5–8	5–6	6–8	5–6	6–8.5
Wing length (mm)	18–18.5	14–18	10–11	16–18	15–16	14–19
Wing limb width (mm)	4–4.2	2–3	2.5–3	3–4	3.3–5	2.8–4
Keel length (mm)	12–13	10–14	7.5–8.5	11–14	13–14	11.5–16
Keel limb width (mm)	2–2.2	1.8–2.5	1.5–1.8	2–2.5	1.8–2	2–2.6
Staminal tube (mm)	11–12	10–13.5	7–8	9–12	11–12	10–15
Pistil length (mm)	13–14	9–12	6–7	9–10	?	9–13
Pod length incl. beak (mm)	16–18	13–26	9–10	18–26	10	16–22
Pod beak length (mm)	10–12	9–19	5.5–6	12–19	5	11–17
Pod beak indumentum	sparsely hairy	pubescent	glabrous	pubescent	pubescent	glabrous
Seed length (mm)	5.5–6	5–7	3.5–4	5–7	5	4.5–5.5
Seed width (mm)	4.5–4.8	3–4	2–2.2	3–4	?	2.4–2.6
Ecology	terricolous	terricolous	terricolous	chasmophilous	chasmophilous	chasmophilous

was decided to select a neotype using the materials available in literature (iconographies) or herbaria (specimens), fully coherent with the protologue. Given that, the choice fell on a colour illustration published by Reichenbach & Beck de Mannagetta in 1903 (Tab. 140 I, MMCXCI), which was surely based on material coming from the "*locus classicus*". Besides, to support this iconotype, an epitype was designated, represented by a herbarium specimen recently collected in Istria by some of the authors.

Other specimens examined – **Croatia**: Istria, eastern coast, Kavran village, along the road, 24 Apr. 2015, *Bogdanović & Ljubičić* s.n. (CAT, ZAGR, barcode ZAGR-39431); Istria, north of Vela Budava bay, road along the forest margin, 24 Apr. 2015, *Bogdanović & Ljubičić* s.n. (ZAGR, barcode ZAGR-39432); Croatia, Istria, northeast of Vela Budava bay, road along the forest, 24 Apr. 2015, *Bogdanović & Ljubičić* s.n. (ZAGR, barcode ZAGR-39433); NP Krka, uz asfaltnu cestu iznad Roškog slapa, 5 Jun. 2015, *Šegota* s.n. (ZAGR, barcode ZAGR-39868); Dalmatinska Zagora, Vrgorac, Duboka draga kod sela Prapatnica, uz cestu, 29 May 2015, *Bogdanović, Vitasović Kosić & Vukojević* s.n. (ZAGR, barcode ZAGR-39869); Dalmacija, Zadar, ruderalno stanište, uz put kod napuštene tvornice, 6 Jun. 2015, *Ljubičić* s.n. (ZAGR, barcode ZAGR-39800); Dalmacija, na Monte Marjanu uz gromače u vinogradima i maslinicima brojno. Oko Sv. Jere i drugdje po vrhu, 13 Jun. 1908, *Hirc* s.n. (ZA); Rijeka, s.d., no collector s.n. (ZA); Dalmatia, Spalato, s.d., no collector s.n. (ZA); Flora Dalmatica: Monte Marian supra Spalato, 12. May 1910, *Lengjel* s.n. (ZA); Dalmatia, 1875, *Vukotinović* s.n. (ZA); Dalmacija, Split, Marjan, zapuštena mjesta prema Sv. Jeri, 4 Jun. 1962, *Trinajstić* s.n. (CNHM, barcode CNHM-32185).

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